Remarks

Applicant and the undersigned would like to thank the Examiner for her efforts in the examination of this application. Reconsideration is respectfully requested.

I. Specification

The Examiner has objected to the Specification as not containing "a written description of the invention . . . in such full, clear, concise, and exact terms as to enable any person skilled in the art" to practice the invention in its best mode."

This objection is respectfully traversed. Please find enclosed herewith an Affidavit of Professor Hsieh addressing the Examiner's objections. In summary, the experiments for optimizing operating conditions were performed with a glove box having a known CO_2 concentration for determining how alkaline solutions absorb CO_2 under various CO_2 concentrations. The experiments presented in FIG. 6 are with real samples of various respiration rates (various bacterial activities from fresh meat to stale milk) because there are validations of the microrespirometer method with a proven method.

The data of FIG. 4 apply to very dilute alkaline solutions of less than 0.01 M, and illustrate that a steady-state head-space CO_2 concentration can be established if there is a source (respiration) and a sink (diluted alkaline solution < 0.01 M) of CO_2 in an enclosed head space. That is, the respiration and CO_2 absorption of the diluted alkaline solution adjust by itself to a steady-state CO_2 concentration in the head space as long the respiration does not change significantly and the alkaline solution is not completely neutralized. The steady-state CO_2 concentration in the head space is controlled by the

respiration rate; i.e., the higher respiration rate, the higher the steady-state CO_2 concentration will be. The respiration rate then can be determined by simply measuring the CO_2 absorption rate of the alkaline solution at the steady state. Only under such a dilute alkaline concentration can the respiration rate be determined at the μL/h level and within a short period of time (< 1 h).

The method described in the Specification and claimed in the present application has received the acknowledgment of peer review in the attached publications by one or more of the inventors. Exhibit A is a published paper entitled "Determination of Carbon Dioxide Evolution Rates Using a Novel Noninstrumental Microrespirometer," by Y. Hsieh and Y. Hsieh (*J. AOAC International* **83**(2), 277-281, 2000). The method was used to provide data for Exhibits B and C, also published in peer-reviewed journals: "Real-Time Determination of Microbial Activity of Pasteurized Fluid Milk Using a Novel Microrespirometer Method," Z. Ren and Y. Hsieh (*J. AOAC International* **88**(6), 1-6, 2005) and "Comparison of the Real-time Microrespirometer and Aerobic Plate Count Methods for Determination of Microbial Quality in Ground Beef," X. Li and Y. Hsieh (*J. Food Science* **68**(9), 2758-63, 2003).

Therefore, the present invention is believed to be enabling as presented.

II. Rejection of Claims 1-13 under 35 USC 103(a)

The Examiner has rejected Claims 1-13 under 35 USC 103(a) as being unpatentable over Stotzky (*Meth. Soil. Anal.*, 1965).

This rejection is respectfully traversed. Professor Hsieh addresses this rejection in the enclosed Affidavit as well. As stated therein, Stotzky teaches the principle of exhaustive CO_2 absorption by alkaline solution to determine respiration rates. Stotzky's methods require alkaline concentrations greater than 0.3 M to exhaustively (completely) absorb CO_2 in the head space in order to determine respiration rate. The principle of exhaustive CO_2 absorption is completely different from the principle of establishing steady-state CO_2 concentration. No steady-state head space CO_2 concentration can be established by the Stotzky method because it would approach zero as the incubation is prolonged. The methods described by Stotzky have much higher detection limit of CO_2 (500 µL) than that of the present microrespirometer method (1 µL) because of the much higher alkaline concentration employed by Stotzky.

Further, the method described by Stotzky requires complete (exhaustive) absorption of head-space CO_2. That is why it must use a much stronger alkaline solution (> 0.3 M) to exhaustively absorb the CO_2. Any substantial CO_2 remaining in the head space of the Stotzky methods contributes error. There is no mention of establishing steady-state CO_2 concentration in the head space by Stotzky because it should not and cannot. The "steady-state equilibrium" described in Stotzky is referring to the respiration of a soil sample that is established by the conditions of water content, temperature, aeration rate, and spatial arrangement of a soil sample (please see the discussion in p. 1553 of the Stotzky paper). The "steady state" of the microrespirometer is referring to the CO_2 concentration in the head space that is established by the balance of CO_2 evolution (respiration) and CO_2 absorption of the diluted alkaline solution. The principles of this invention and that of Stotzky's are fundamentally different. The acid used in the titration

of the microrespirometer method is the CO_2 respired by the sample rather than by a HCl solution as described by Stotzky. A comparison of the microrespirometer method and that of the Stotzky is summarized in the table contained in the enclosed Affidavit.

This invention is different from all the current respirometer methods in that it does not rely on the exhaustive absorption of CO_2, as in the case of Stotzky, nor on the measurement of the CO_2 concentration (such as the IR analyzer methods) in the head space to estimate the respiration rate. Exhaustive absorption of CO_2 in the head space requires much concentrated alkaline solution (at least 0.5 M vs. 0.001 M used in this invention). Higher concentrations of alkaline solution reduce the sensitivity of a method because the smallest CO_2 unit can be detected is the product of (concentration of alkaline solution)* (smallest unit of titration). Methods that use exhaustive absorption CO_2 thus have a detection limit = 0.5 M * 0.1 mL = 50 μM CO_2 or 1120 μL CO_2, a rather large value of CO_2 in comparison to the level that is measured by this invention.

The detection limit of this invention is approximately 500 times better than the exhaustive CO_2 absorption methods because it uses 500 times less diluted alkaline solution, i.e., 0.001 M * 0.1 mL = 0.1 μM CO_2 or 2.2 μL CO_2. The principle used in the invention, therefore, cannot be the exhaustive absorption of CO_2. Rather, it relies on the steady-state CO_2 concentration maintained by the CO_2 evolved from a sample and the CO_2 absorbed by the weak alkaline solution. That is how the pre-incubation comes into play in the invention. During the pre-incubation, the head space CO_2 concentration either will go up, if the respiration rate exceeds the CO_2 absorption rate of the alkaline solution, or it will go down, if the respiration rate is less than the absorption rate of the alkaline solution. The change of CO_2 concentration in the head space will slow down according to FIG. 4

until a steady-state CO_2 concentration is established and maintained by the balance of the respiration and alkaline CO_2 absorption. At this steady-state, the respiration rate equals the CO_2 absorption rate. The respiration rate then can be determined by measuring the CO_2 absorption rate (via an indicator).

The uniqueness of this invention includes the elimination of the need for a strong alkaline solution (exhaustive absorption of CO_2). Instead this invention requires a very weak alkaline solution to maintain a steady-state CO_2 concentration in the head space for the respiration rate assessment. The detection limit of this invention is about 500 times more sensitive than that of Stotzky, and the time required is also much shorter (1 h for the present invention vs. > 6 h of Stotzky). The method taught by Stotzky cannot measure what the present invention can.

Conclusions

Applicants respectfully submit that the above amendments place this application in a condition for allowance, and passage to issue is respectfully solicited. Applicants and the undersigned would like to again thank the Examiner for her efforts in the examination of this application and for reconsideration of the claims as amended in light of the

arguments presented. If the further prosecution of the application can be facilitated through telephone interview between the Examiner and the undersigned, the Examiner is requested to telephone the undersigned at the Examiner's convenience.

Respectfully submitted,

Jacqueline E. Hartt, Ph.D.
Reg. No. 37,845
ALLEN, DYER, DOPPELT, MILBRATH & GILCHRIST, P.A.
255 South Orange Avenue, Suite 1401
P.O. Box 3791
Orlando, Florida 32802
(407) 841-2330
Agent for Applicant

CERTIFICATE OF MAILING

I hereby certify that the foregoing is being deposited with the United States Postal Service as first class mail in an envelope addressed to the Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, this 3rd day of March, 2006.

Edward Bradley